

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)



EUROPEAN PATENT APPLICATION

Application number : **94301820.0**

Int. Cl.⁶ : **G01N 35/08, G01N 1/00, B01L 3/00**

Date of filing : **15.03.94**

Priority : **16.03.93 JP 55327/93**

Inventor : **Kamahori, Masao**
Hitachi Hatoyamaryo,
2527, Akanuma
Hatoyamamachi, Hiki-gun, Saitama-ken (JP)

Date of publication of application : **21.09.94 Bulletin 94/38**

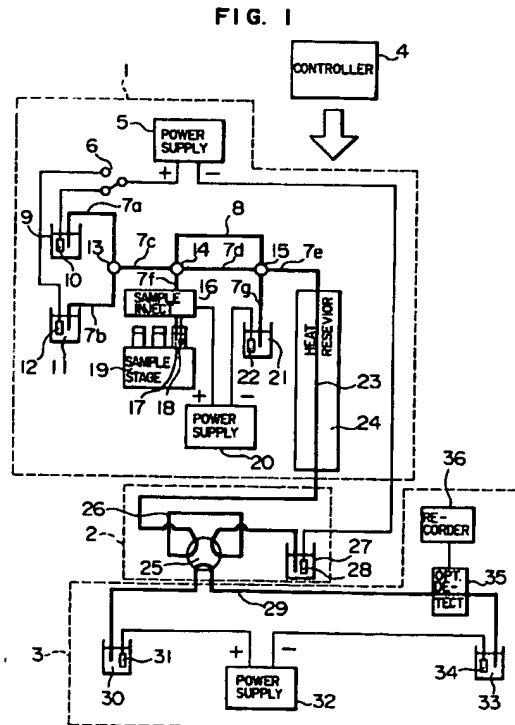
Designated Contracting States : **DE FR NL**

Representative : **Calderbank, Thomas Roger et al**
MEWBURN ELLIS
York House
23 Kingsway
London WC2B 6HP (GB)

Applicant : **HITACHI, LTD.**
6, Kanda Surugadai 4-chome
Chiyoda-ku, Tokyo 101 (JP)

54 Micro-reactor device and minute sample analysis system using the same.

57 A minute sample analysis system which comprises a micro-reactor device (1), a quantitative measuring device (2), a analyzing device (3) and a controller (4), whereby, when a very small amount of sample is handled, its dilution and loss can be suppressed to minimum level and analyzing operations ranging from reaction with reactive reagent to separation/detection of the sample can be consistently carried out efficiently. The micro-reactor device (1) controls the solution, reactive reagent and sample flowing in the form of electroosmotic flow generated by high-voltage application under control of passage change-over switches; while the quantitative measuring device (2) measures the quantity of reactive sample received from the micro-reactor device (1) and introduces the measured reactive sample into the analyzing device (3). The analyzing device (3) optically detects components separated from the sample through electrophoresis. The above operations are generally controlled under the controller (4).



The present invention relates to a micro-reactor device in which a minute of sample material is made to react in a microscopic area and also to a minute sample analysis system which uses the micro-reactor device.

As a method for causing reaction between sample and reactive reagent on a flow basis, a flow injection analysis is general wherein sample is introduced into reactive reagent and made to react therewith during flow of the sample liquid to be subjected to a concentration measurement by an optical detection method based on its absorbance, which details are shown, for example, in Analytical Chemistry, Vol. 50(1978), pp. 832A-846A or in Analytical Chemistry, Vol. 53(1981), pp. 20A-32A or in Analytica Chimica Acta, Vol. 78(1975), pp. 145-157.

In the case where such a liquid feeding pump of a mechanical drive type as used in the above-mentioned flow injection analysis, flow within a flow passage becomes such laminar flow having a flow profile 41 as shown in Fig. 2. The laminar flow has such a velocity distribution that the flow has a velocity of substantially zero at its both ends due to the flow resistance of walls 42 and 43 of the passage and has a maximum velocity at its central part. For this reason, there occurs a problem that such a difference in the flow velocity within the passage causes the injected sample to flow through the passage without keeping its original shape. And consequently, band broadening of the injected sample as a result of mixing with the solution at its front and rear ends thereof, results in a decrease of concentration of the sample liquid and in an increase of volume in the sample.

In this connection, a pressure drop Δp is expressed as a Hagen-Poiseuille law which follows.

$$\Delta p = 8\mu Q/\pi r^4$$

where μ denotes the viscosity of the liquid, l denotes the length of the passage, Q denotes flow quantity, and r denotes the radius of the passage.

That is, the pressure drop increases inversely proportional to the fourth power of the radius of the passage. For this reason, when a capillary as small as below $100\mu\text{m}$ is used as the passage for the purpose of handling such a very small amount of sample as nanoliter level, the pressure drop becomes large, which involves another problem of withstanding pressure within the apparatus, that is, which requires a special measure of providing a pressure resistive property to the wall material of the passage and also to a coupling part between the passages to be taken.

Thus, there have not been so far realized a micro-reactor device wherein a very small amount of sample as minute as nanoliter level is made to react with reactive reagent as well as a minute sample analysis system which is a combination of the micro-reactor device as its pretreatment and a analyzing device suitable for analysis of a very small amount of sample composition such as a capillary electrophoresis de-

vice.

In order to solve the above problems, in accordance with the present invention, transfer of sample and reactive reagent in a micro-reactor device is carried out on an electroosmotic flow.

Further, the micro-reactor device is formed on a planar substrate having very narrow grooves.

Furthermore, the micro-reactor device is coupled via a quantitative measuring device with a capillary electrophoresis device.

Electroosmotic flow takes place when application of a voltage across a capillary tube causes electric double layers 51 and 52 formed on the internal surface of the tube to move in the same direction as an electric field established by the applied voltage, as shown in Fig. 3. In this case, a flow profile 53 is such a flat flow as shown in Fig. 3. For this reason, sample diffusion is as small as several tenths of that in the case of laminar flow. A velocity u_{osm} of the electroosmotic flow is expressed by the following equation.

$$u_{osm} = k\epsilon E/z\eta\sqrt{c}$$

where, k denotes a constant, e denotes charge quantity of the capillary tube per its unit surface, E denotes applied voltage, z denotes the number of charges in electrolyte, η denotes the viscosity of solution, and c denotes the concentration of the electrolyte.

In this way, since the electroosmotic flow depends on the applied voltage, the concentration of the electrolyte in the solution, the sign and the quantity of charges on the surface of the capillary tube, control of the quantity of solution to be transferred can be facilitated. Further, the pressure drop caused by the solution transfer is substantially zero.

The capillary electrophoresis is an effective analyzing method having a high separation ability but requires a sample quantity to be as very small as nanoliter level. Thus, for the purpose of preventing a large quantity of sample solution from being introduced from the micro-reactor device into the capillary electrophoresis device, there is provided a quantitative measuring device between the capillary electrophoresis device and the micro-reactor device. As a result, a very small amount of sample can be accurately introduced into the capillary electrophoresis device, and on-line analysis including the reaction of a very small sample with the reagent and separation of sample composition can be performed without subjecting to any dilution and loss.

In the drawings:

Fig. 1 is a block diagram of an arrangement of a minute sample analysis system in which a first micro-reactor device is used in accordance with the present invention;

Fig. 2 shows a flow profile of laminar flow;

Fig. 3 is a flow profile of electroosmotic flow;

Figs. 4A and 4B show detailed steps in a reagent

introduction method;

Figs. 5A, 5B and 5C show detailed steps in a sample introduction method and in a sample-reagent reaction method;

Figs. 6A and 6B show detailed steps in an analysis method;

Fig. 7 is a block diagram of an arrangement of a second micro-reactor device in accordance with the present invention;

Figs. 8A and 8B show a structure of flow passages of the second micro-reactor device; and

Figs. 9A and 9B show a structure of a passage switching part in the second micro-reactor device.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A first embodiment of the present invention will be explained with reference to Fig. 1 showing its block diagram.

A minute sample analysis system of Fig. 1 in accordance with the first embodiment of the present invention comprises a micro-reactor device 1, a quantitative measuring device 2, an analyzing device 3, and a controller 4.

More specifically, the micro-reactor device 1 includes a power supply 5 for liquid transfer; power change-over switch 6; passages 7a to 7g; sample quantity measurer 8; a solution resevoir 9; a reactive reagent resevoir 11; platinum electrodes 10, 12, 18 and 22; passage change-over switches 13, 14 and 15; an automatic sample injector 16, a sample resevoir 17, a sample stage 19, a power supply 20 for sample introduction, a waste solution resevoir 21, a reactor 23, and a constant-temperature heat resevoir 24. The micro-reactor 1 functions to provide pre-treatment to cause reaction between sample and such reactive reagent as fluorescent reagent.

The power supply for liquid transfer 5, which comprises a high voltage power supply having an output voltage of 0-30kV, applies a high voltage to between the platinum electrode 10 of the solution resevoir 9 and the platinum electrode 28 of the waste solution resevoir 27 of the quantitative measuring device 2 or to between the platinum electrode 12 of the reactive reagent resevoir 11 and the platinum electrode 28 of the waste solution resevoir 27 of the quantitative measuring device 2. An eluting solution within the solution resevoir 9, when the high voltage is applied to between the solution resevoir 9 and the waste solution resevoir 27 of the quantitative measuring device 2, is circulated in the form of an electroosmotic flow caused by the high voltage application through the passages 7a, 7c, 7d and 7e sequentially in this order. Similarly, a reactive reagent solution within the reactive reagent resevoir 11, when the high voltage is applied to between the reactive reagent resevoir 11 and

the waste solution resevoir 27 of the quantitative measuring device 2, is circulated in the form of an electroosmotic flow caused by the high voltage application through the passages 7b, 7c, 7d and 7e sequentially in this order.

The flows of the above eluting and reactive reagent solutions can be controlled with use of the passage change-over switches 13, 14 and 15. Their flow rate can be easily set by controlling the applied voltage. In more detail, the power change-over switch 6 acts to select the voltage application between the solution resevoir 9 and the waste solution resevoir 27 of the quantitative measuring device 2 or the voltage application between the reactive reagent resevoir 11 and the waste solution resevoir 27 of the quantitative measuring device 2. By controlling the applied voltage and the switching time, the amount of reactive reagent introduced into the passages can be readily adjusted. In this connection, each of the passages 7a to 7e was made up of a glass capillary tube (manufactured by GL Sciences company) having an inner diameter of 75 μ m and an outer diameter of 375 μ m. Further, the passage change-over switches 13, 14 and 15 may be replaced, for example, by a three-way valve.

Sample introduction to the sample quantitative measurer 8 is carried out by means of the power supply 20 for sample introduction applying a high voltage to between the platinum electrode 18 of the sample resevoir 17 placed on the sample stage 19 and the platinum electrode 22 of the waste solution resevoir 21. First of all, the automatic sample injector 16 is used to insert a tip end of the passage 7f into the sample resevoir 17 placed on the sample stage 19. Thereafter, the high voltage is applied to between the platinum electrode 18 of the sample resevoir 17 and the platinum electrode 22 of the waste solution resevoir 21 so that the sample solution within the sample resevoir 17 flows in the form of an electroosmotic flow caused by the high voltage application through the passages 7f, 8 and 7g sequentially in this order. In this case, the amount of sample solution introduced can be set by the volume (internal volume) of the sample quantitative measurer 8. The tip end of the passage 7f and the platinum electrode 18 are assumed to be moved together by the sample stage with respect to the respective samples placed thereon.

Even when the sample quantitative measurer 8 is not used, the amount of sample solution introduced can be easily controlled by adjusting the applied voltage and application time. More specifically, by suitably switching the passage change-over switches 14 and 15 so as to communicate with the passages 7f, 7d and 7g, the magnitude and application time of the high voltage applied from the power supply for sample introduction 20 to between the platinum electrodes 18 and 22 can be adjusted.

Thereafter, the introduced sample solution sent

through the passage 7e to the constant-temperature resevoir 24, made to react within the reactor 23 of the resevoir 24 with the reactive reagent sent from the reactive reagent resevoir 11, and then sent to the quantitative measuring device 2. In this case, the constant-temperature resevoir 24 is kept at an optimum temperature for the reaction.

The quantitative measuring device 2 includes a passage change-over unit 25, the reacted sample quantitative measurer 26, the waste reactive solution resevoir 27 and the platinum electrode 28; and functions to perform quantitative measuring operation over the reaction sample subjected to the reaction at the micro-reactor device 1 and then to supply the quantitative-measured sample to the analyzing device 3.

The analyzing device 3 as a capillary electrophoresis device in the present embodiment includes a capillary tube 29, a buffer resevoir 30, a buffer waste solution resevoir 33, platinum electrodes 31 and 33, a power supply for analysis 32, an optical detector 35 and a recorder 36. In this case, used as the capillary tube 29 was a glass capillary tube (manufactured by GL Sciences company) having an inner diameter of 75 μ m and an outer diameter of 375 μ m.

First of all, the power supply for analysis 32 is used to apply a high voltage to between the platinum electrode 31 of the buffer resevoir 30 and the platinum electrode 34 of the buffer waste solution resevoir 33 to thereby provide preliminary electrophoresis to solution and to keep the solution in such an electrophoresis enable state. After that, the reacted sample within the reacted sample quantitative measurer 26 of the quantitative measuring device 2 is introduced into the capillary tube 29 for electrophoresis. Components of the reacted sample separated within the capillary tube 29 by the electrophoresis are detected by the optical detector 35 and the migration times and concentration values for the respective detected components are sent to the recorder 36 to be recorded therein.

Although the capillary electrophoresis device has been used as the analyzing device in the present embodiment, a high performance liquid chromatography device may be employed in place of the capillary electrophoresis device while not compelling great modification in the device arrangement.

Further, since such operations as mentioned above are controlled by the controller 4, when the applied voltage and time, the power change-over timing, the passage change-over timing, etc. are controlled in the form of a computer program, this control can be carried out with use of a single switch.

The detailed procedure of a change-over method between the solution and reactive reagent will be explained by referring to Fig. 4 showing a part of the micro-reactor device 1 in Fig. 1.

First of all, when it is desired to supply the solu-

tion, a power supply 61 for sample introduction is operated to apply a high voltage to a solution resevoir 63, in which case a power change-over switch 62 operatively connected with a passage change-over switch 65 is set at such a position as to form a thick solid line passage shown in Fig. 4A. Next, when it is desired to supply the reactive reagent, the power change-over switch 62 is switched to the other position so that, at the same time that a high voltage is applied to a reactive reagent resevoir 64, the passage change-over switch 65 operatively connected with the power change-over switch 62 is also switched, whereby such a path as shown by a thick solid line in Fig. 4B is established. In this case, passage change-over switches 66 and 67 are operatively connected with the power supply for sample introduction 61, so that, when it is desired to supply the solution by means of the power supply for sample introduction 61, such a path as shown by a thick solid line in Fig. 4B is formed.

The detailed procedures of a sample introducing method and a reaction method between the sample and reactive reagent will be explained by referring to Fig. 5 showing a part of the micro-device 1 in Fig. 1.

When it is desired to introduce the sample as shown in Fig. 5A, an automatic sample injector 73 is operated to insert a tip end of a passage 72a into a sample resevoir 75 placed on a sample stage 74, and then a power supply 77 for sample introduction is operated to apply a high voltage to between the sample and waste solution resevoirs 75 and 76. Application of the high voltage to the sample and waste solution resevoirs 75 and 76 causes generation of an electroosmotic flow, whereby the sample solution within the sample resevoir 75 flows through passages 72a, 71 and 72b sequentially in this order. At this time, the reactive reagent is also being supplied through passages 78a, 78b and 87c sequentially in this order. In other words, as shown in Fig. 5B, there are reactive reagents 80 and 81 at upstream and downstream or front and rear ends of a sample 79, that is, the sample is put in a sandwiched relation between the reactive reagents 80 and 81. Thereafter, supply of the solution by the electroosmotic flow causes the sample and reagents to flow while reacting with one another as shown in Fig. 5C. Further, since the sample 83 is put in the sandwiched relation between the reactive reagents 82 and 84 to be efficiently mixed with the reactive reagents 82 and 84 at the front and rear ends of the sample 83 through diffusion, the efficient reaction can be realized. As already explained above, the passage change-over switches 66 and 67, when it is desired to supply the solution by means of the operation of the power supply for sample introduction 61, are set at such positions as to form the path shown by the thick solid line in Fig. 4B. However, when it is desired to introduce the sample, power change-over to the power supply for sample introduction 77 causes

change-over of the passage change-over switches 66 and 67, with the result that such a path as shown by a thick solid line in Fig. 5A is formed.

Explanation will be made as to the more detailed procedure of a method for analyzing the reactive sample in connection with Fig. 6 showing a part of the quantitative measuring device 2 and analyzing device 3 in Fig. 1.

First, for the purpose of providing preliminary electrophoresis, a power supply for analysis 95 is operated to apply a high voltage to between a buffer reservoir 94 and a buffer waste solution reservoir 96. At this time, as shown in Fig. 6A, the reacted sample supplied from the micro-reactor device 1 is filled with a reacted sample quantitative measurer 92 of a passage change-over switch 91. Thereafter, the passage change-over switch 91 is switched so that the reacted sample is introduced into a capillary tube 93 for electrophoresis as shown by a thick solid line in Fig. 6B. In this connection, the passage change-over switch 91 is operatively connected with an optical detector 97 and a recorder 98 so that change-over of the switch 91 causes simultaneous analysis and recording of the sample thereat.

Since the transfer of the sample and reactive reagent is based on electroosmotic flow in the present embodiment, the diffusion of the sample and reactive reagent is as very small as several tenths of that in the case of laminar flow. Further, substantially no pressure drop can be caused by the solution transfer and the reaction between a very small amount of sample and reactive reagent can be efficiently carried out within such a capillary tube as small as below 100 μ m in inner diameter. Furthermore, since the micro-reactor device is connected via the measuring device to the capillary electrophoresis device, a very small amount of sample can be accurately introduced into the capillary electro-phoresis device and on-line analysis including reaction of the very small amount of sample with the reagent and separation of sample composition can be performed without involving any dilution and loss of the sample.

In the foregoing embodiment, explanation has been made in connection with such a system that is an integral combination of the micro-reactor device, measuring device and capillary electrophoresis device. Thus, when the micro-reactor device alone is extracted from the system, one terminal for supplying power to provide electroosmotic flow is missing in the micro-reactor device but this problem can be solved by providing a reservoir corresponding to the waste solution reservoir 27 of the quantitative measuring device 2 to the micro-reactor device.

Explanation will be made as to a micro-reactor device in accordance with a second embodiment of the present invention by referring to Fig. 7 showing its block diagram.

The illustrated micro-reactor device of the sec-

ond embodiment includes power supplies 101 and 102, a reactive reagent reservoir 103, waste solution reservoirs 104 and 105, sample reservoirs 106a to 106d, passages 107a to 107f, passage change-over switches 108, 109, 110, 111, 112, 113 and 114, a measurer 115, a light source 116, a detector 117, and a controller 118. The micro-reactor device except the power supplies is formed on a planar plate insulator such as a glass plate, a single crystal silicone substrate, etc.

In more detail, the power supply 102 having a high output voltage of 0-30kV is used to apply a high voltage to between an electrode of the reactive reagent reservoir 103 and an electrode of the waste solution reservoir 104. The power supply 101 is used to apply a high voltage to between electrodes of the sample reservoirs 106a to 106d and an electrode of the waste solution reservoir 105.

When the high voltage is applied to between the electrode of the reactive reagent reservoir 103 and the electrode of the waste solution reservoir 104, the electroosmotic flow generated by the high voltage application causes the reactive reagent within the reactive reagent reservoir 103 to flow through the passages 107a, 107b and 107c sequentially in this order. Similarly, when the high voltage is applied to between the electrodes of the sample reservoirs 106a to 106d and the electrode of the waste solution reservoir 105, the electroosmotic flow generated by the high voltage application causes the sample solution within the sample reservoirs 106a to 106d to flow through the passages 107d, 107e, 107b and 107f sequentially in this order. In the illustrated example, the micro-reactor device is designed for selective application of 4 samples. The flows of the above reactive reagent and sample can be switchingly controlled by means of the passage change-over switches 108, 109, 110 and 111 controlled based on a signal issued from the controller 118. In this connection, the flow rate can be easily set by adjusting the applied voltage or time of the power supplies 101 and 102 on the basis of a signal from the controller 118.

The reaction of the micro-reactor device of the present embodiment is carried out in the following sequence.

First of all, the reactive reagent is introduced into the passages 107a, 107b and 107c, at which time the passage change-over switches 110 and 111-114 are operated to close the path and to stop the flowing of the sample. Subsequently, a high voltage is applied to between the electrode of the reactive reagent reservoir 103 and the electrode of the waste solution reservoir 104 so that the electroosmotic flow generated by the high voltage application causes the reactive reagent within the reactive reagent reservoir 103 to flow through the passages 107a, 107b and 107c sequentially in this order.

Thereafter, the passage change-over switches

108 and 109 are operated to close the path and to stop the flowing of the reactive reagent.

Next, when it is desired to introduce the sample into the passage 107b also functioning as a sample quantitative measurer, the power supply 101 for sample injection is operated to apply a high voltage to between the electrode of the sample reseviior 106a and the electrode of the waste solution reseviior 105.

The passage change-over switches 110 and 111 are first operated to open the path. After that, a high voltage is applied to between the electrode of the sample reseviior 106a and the electrode of the waste solution reseviior 105 so that the electroosmotic flow generated by the high voltage application causes the sample within the sample reseviior 106a to flow through the passages 107d, 107e, 107b and 107f sequentially in this order. In this conjunction, the amount of sample introduced can be set by the capacity of the passage 107b functioning also as a sample quantitative measurer. Even with respect to the sample solutions of the sample reseviiors 106b to 106d, the sample introduction can be similarly controlled by the passage change-over switches 112, 113 and 110.

With respect to the introduced sample and reactive reagent, the passage change-over switches 110 and 111 are operated to close the path and to stop the flowing of the sample and subsequently the passage change-over switches 108 and 109 are operated to open the reactive reagent path. Under this condition, when the high voltage is applied to between the electrode of the reactive reagent reseviior 103 and the electrode of the waste solution reseviior 104, the electroosmotic flow generated by the high voltage application causes the sample and reactive reagent to flow through the passages 107b and 107c while reacting with each other. Thus, there are reactive reagents at the front and rear ends of the sample introduced into the passage 107b, that is, the sample is put in a relationship sandwiched between the reactive reagents. Thereafter, the solution transfer based on the electroosmotic flow causes the sample and reactive reagent to react with each other while flowing. At this time, since the sample is sandwiched between the reactive reagents, the sample can be efficiently mixed with the reactive reagents at the front and rear ends thereof through diffusion for efficient reaction therebetween. When the optimum temperature of the reaction is high, temperatures in the passages 107b and 107c can be set at proper levels for reaction without any troubles.

After that, light from the light source 116 is directed to the reacted sample. Change of light intensity due to the reacted sample is detected by the detector 117 to measure a sample quantity. In this connection, the change of light intensity means that of absorbance, fluorescence intensity, etc. Thus, the measurer 115 has a high light transmittance and especially in case of absorbance change measurement, the

measurer passage is provided thereon with a light reflecting layer to prolong its light path length. Further, when it is desired to measure a multiplicity of samples, this can be easily realized by sequentially operating the passage change-over switches 111, 112, 113 and 114 in the similar procedure to the above.

The aforementioned operations are controlled by the controller 118 and thus when the applied voltage and time, passage change-over timing, etc. are controlled in accordance with a computer program, the operation control can be realized with use of a single switch.

More detailed explanation will be made as to the passage arrangement of the aforementioned micro-reactor device by referring to Fig. 8.

Fig. 8A shows a passage arrangement of the micro-reactor device. The passages of the micro-reactor device are formed by first providing very narrow grooves and small through holes in such a planar substrate as a glass or silicon substrate, overlapping another planar substrate on the former substrate, and then joining the substrates together by fusion bonding. As a result, passages 141a to 141h are defined by the very narrow grooves while a reactive reagent reseviior 142, waste solution reseviiors 143 and 144, and sample reseviiors 145a to 145d are defined by the small through holes. The formation of the very small grooves and small through holes may be effected by such a mechanical machining with use of a drill or by such a chemical treatment as etching. Further, passage change-over switches 146a to 146g may function to perform their switching operation by mechanically opening or closing the small through holes for passage change-over or by partially freezing or unfreezing the passages 141a to 141h.

Fig. 8B shows a side cross-sectional view of the micro-reactor device of Fig. 8A as viewed from a passage position A-A shown by arrows. In the drawing, reference numeral 200 denotes a planar substrate which is provided in its one surface with very small grooves and small through holes. Numeral 300 denotes a planar substrate overlapped on the substrate 200. The passage change-over switches 146a and 146c are provided therein with members 146a' and 146c' which function as stop plugs and as already explained above, which are controlled by the controller 118 to open or close the associated passages. Further, the reactive reagent reseviior 142, waste solution reseviiors 143 and 144, and sample reseviiors 145a to 145d are provided on their walls with electrodes for providing electroosmotic flow (only two of which electrodes for the reactive reagent reseviior 142 and waste solution reseviior 144 being illustrated in the drawing).

Since the reactive reagent reseviior 142, waste solution reseviiors 143 and 144, and sample reseviiors 145a to 145d are provided in the same planar substrate in the present embodiment, the need for con-

necting the reactive reagent resevoir, waste solution resevoirs and sample resevoirs through connectors as in the prior art can be eliminated and thus a leakage problem and the need for interconnections in very small areas can be removed. Further, since only the controller, high voltage power supplies and optical detector are provided as external devices, the entire apparatus can be made easily small in size.

Furthermore, since the reactive reagent resevoir 142, waste solution resevoirs 143 and 144, and sample resevoirs 145a to 145d are disposed as externally faced, the introduction and the exchange of the reactive reagent and sample, the washing, and the waste solution removing can be facilitated. In this connection, the amounts of reactive reagent and sample used depend on the sizes of the reactive reagent resevoir and sample resevoirs. For this reason, minute amount of sample as very small as microliter level can be exchanged without any loss by making the diameter of the small through holes for the reactive reagent resevoir and sample resevoirs to be below 5000 μ m. A measurer 147 includes a light transmittable part 148 made of silica glass having a high light transmittance and a light reflecting layer 149. The light reflecting layer 149 is made preferably of such material having an excellent reflectance as platinum or rhodium. When it is desirable to provide the measurer in the form of a light transmission type, the reflecting layer 149 can be omitted.

Explanation will be made as to an example of the structure of a passage change-over means by referring to Fig. 9.

Fig. 9A shows a part of the passage change-over means which includes sample passages 151a and 151b, reactive reagent passages 152a to 152c and passage change-over switches 153 and 154. In this case, the passage 152b functions also as a sample quantitative measurer. The sample quantitative measurement and reaction can be carried out by closing the passage change-over switches 153 and 154 to introduce the sample into the passage 152b functioning also as the sample quantitative measurer. Fig. 9B shows a side cross-sectional view of a part of a passage change-over means which includes Peltier elements 158, 159, 160 and 161 which are made in planar substrates 156 and 157 as opposed to each other with a passage 155 disposed therebetween. Passage change-over can be effected by cooling the solution in the passage to -15°C or less by means of the Peltier elements 158, 159, 160 and 161 to close the passage 155.

According to the present embodiment, the passage change-over in microscopic areas can be facilitated with a simple arrangement because the opening and closing of the passages is carried out by freezing and unfreezing the solution in the passages.

Claims

1. A micro-reactor device (1) for causing reaction between sample and reactive reagent in a very small area, wherein the sample and reactive reagent are transferred using electroosmosis as the driving force.
2. A micro-reactor device (1) for causing reaction between sample and reactive reagent within a capillary tube having an inner diameter of 100 μ m or less, wherein application of a voltage to parts of said capillary tube causes the sample or reactive reagent to be transferred using electroosmotic flow generated by said application of the voltage as the driving force.
3. A micro-reactor device (1) comprising:
 - at least two capillary tubes (7a-7g) having an inner diameter of 100 μ m or less;
 - a power supply (5, 20) for applying a voltage to parts of said capillary tubes to generate an electro-osmotic flow in the capillary tubes;
 - a power change-over switch (6) for switching said power supply;
 - a passage change-over switch (13, 14, 15) for switching said power supply to a capillary tube to be activated to establish a passage having an electroosmotic flow; and
 - a controller (4) for controlling said power supply, said power change-over switch and said passage change-over switch.
4. A minute sample analysis system wherein a sample and a reactive reagent are transferred on an electroosmotic flow basis as the driving force and a micro-reactor device (1) for causing reaction between the sample and reactive reagent is connected to such an analyzing device (3) as a capillary electrophoresis device or a liquid chromatography device.
5. A minute sample analysis system as set forth in Claim 1, wherein interconnection between said micro-reactor device (1) and said analyzing device (3) is carried out through a quantitative measuring device (2).
6. A minute sample analysis system as set forth in Claims 1 - 5, wherein said system comprises a controller (4) for controlling said micro-reactor device (1), said quantitative measuring device (2) and said analyzing device (3).
7. A minute sample analysis system wherein said micro-reactor device (1) introduces sample and reactive reagent in the form of electroosmotic flow generated when a voltage is applied to parts

of a capillary tube having an inner diameter of 100µm or less to cause reaction between said sample and reactive reagent within said capillary tube.

8. A minute sample analysis system having a micro-reactor device (1), wherein said micro-reactor device comprises:

at least 2 or more of capillary tubes (7a-7g) having an inner diameter of 100µm or less;
a power supply (5, 20) for applying a voltage to parts of said capillary tubes;

a power change-over switch (6) for switching said power supply; and

a passage change-over switch (13, 14, 15) for switching a passage having electroosmotic flow generated when a voltage is applied to parts of said capillary tubes.

9. A micro-reactor device (1) for causing reaction between sample and reactive reagent wherein very narrow grooves having an inner diameter of 100µm or less are formed in a planar substrate (200) made of an insulating material and said sample and reactive reagent are supplied in the form of electroosmotic flow generated when a voltage is applied to parts of said very narrow grooves.

10. A micro-reactor device (1) as set forth in claim 9, wherein said planar substrate (200) having said very narrow grooves of an inner diameter of 100µm or less formed therein is overlapped on a second planar substrate (300) as opposed to said first planar substrate to cause very narrow tubes to be defined by said very narrow grooves.

11. A micro-reactor device (1) comprising:

very narrow branched grooves formed in a substrate (200) made of an insulating material, said grooves having an inner diameter of 100µm or less and being connected to each other;

resevoirs (145a-145d, 142, 143, 144) formed at tip ends of said very narrow branched grooves and having sample, reactive reagent and waste solution contained therein;

a power supply (101, 102) for applying a voltage through the very narrow grooves to between said resevoir containing said sample and said resevoir containing said waste solution or to between said resevoir containing said reactive reagent or waste solution and said resevoir containing said sample to cause the solution within said associated very narrow grooves to be subjected to electroosmotic flow;

passage change-over switches (146a-146g) provided to said very narrow grooves for opening or closing a path of said solution; and

a controller (118) for controlling a voltage application condition of said power supply and said passage change-over switches,

wherein the very narrow grooves between said sample resevoir and said waste solution resevoir and the very narrow grooves between said reactive reagent resevoir and said waste solution resevoir share at least partly a common path.

5

10

15

20

25

30

35

40

45

50

55

8

12. A micro-reactor device (1) as set forth in Claim 11, wherein said very narrow grooves are formed in one side of a substrate (200), said resevoirs include holes passed through said substrate, and a second substrate is overlapped on one side of said first substrate.

13. A micro-reactor device (1) as set forth in Claim 1, wherein said passage change-over switches perform opening and closing control of electroosmotic flow by freezing or unfreezing a part of said very narrow grooves.

14. A micro-reactor device (1) as set forth in Claim 12, wherein said passage change-over switches perform opening and closing control of electroosmotic flow by freezing or unfreezing a part of said very narrow grooves.

FIG. 1

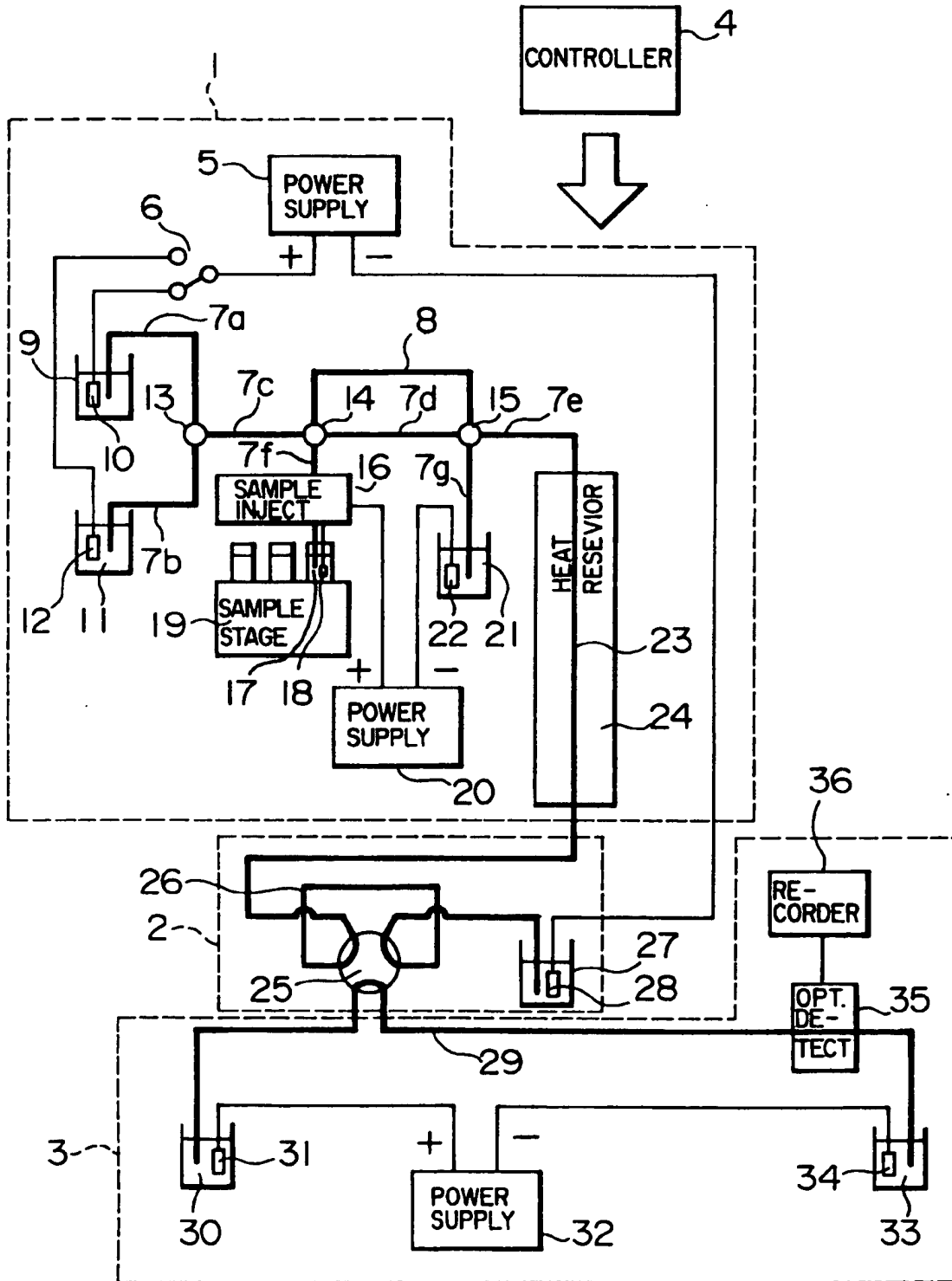


FIG. 2

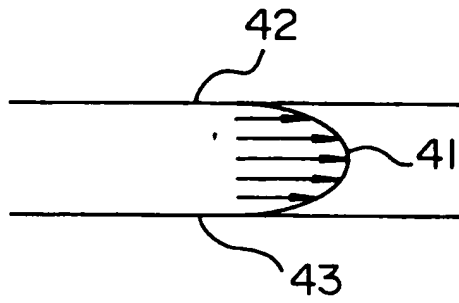


FIG. 3

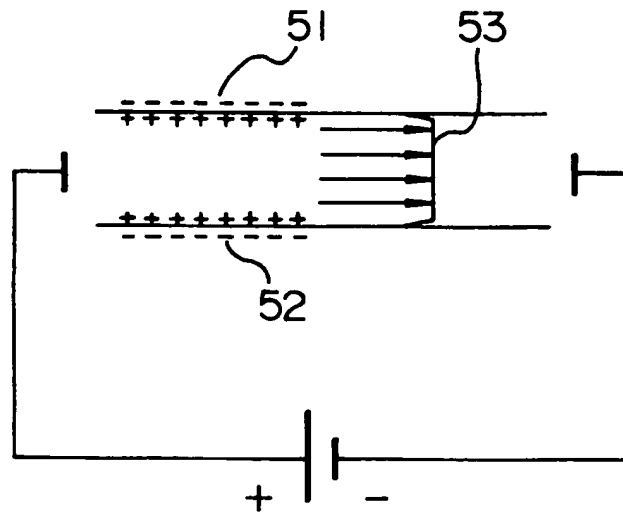


FIG. 4A

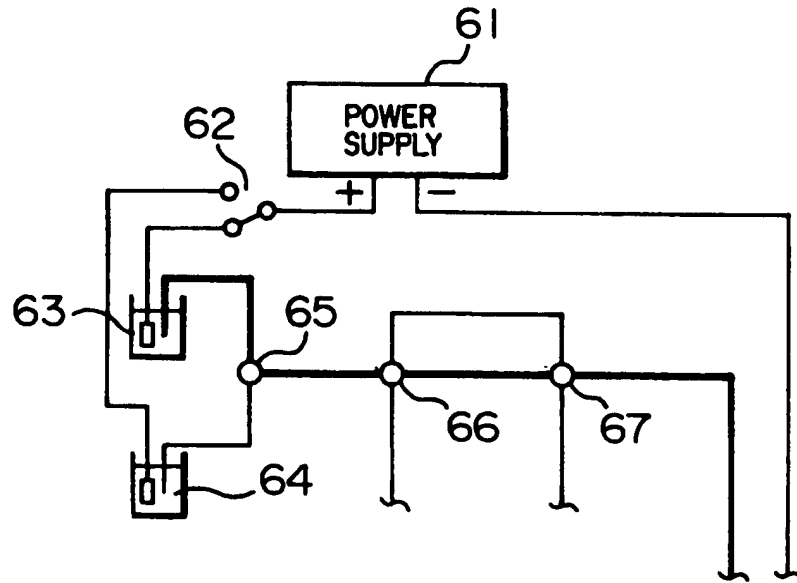


FIG. 4B

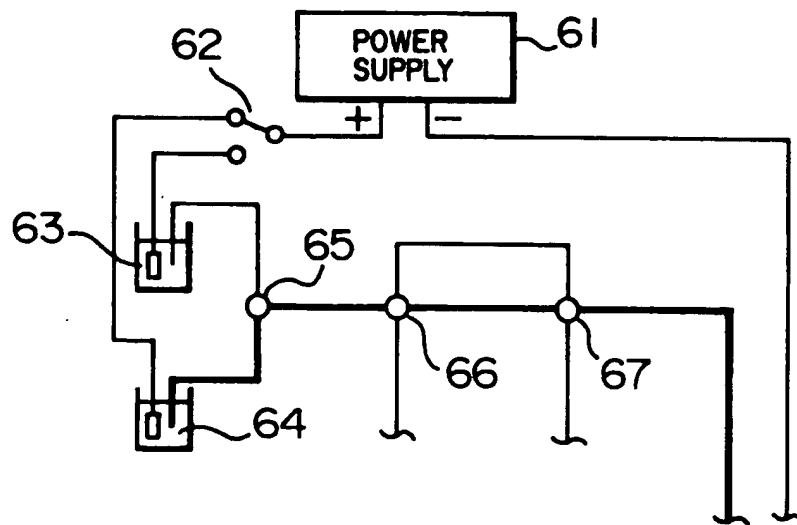


FIG. 5A

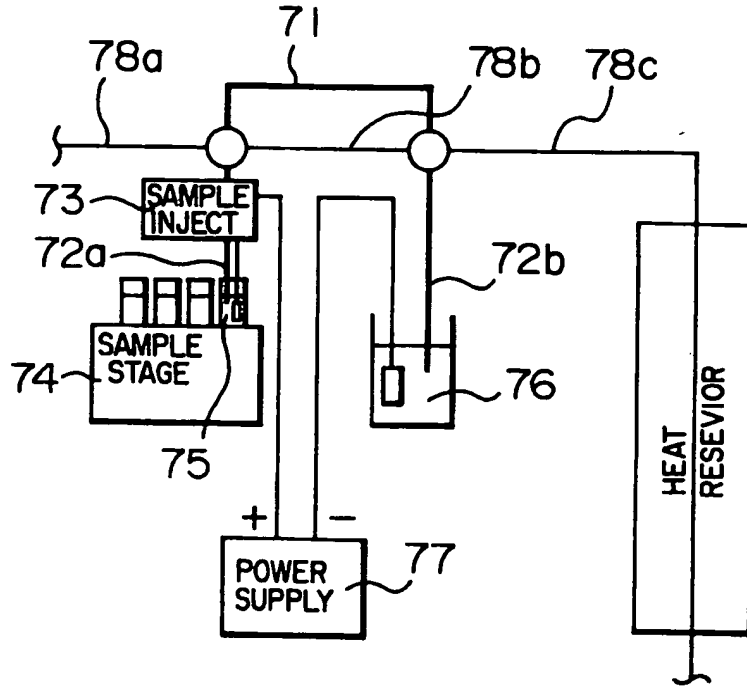


FIG. 5B

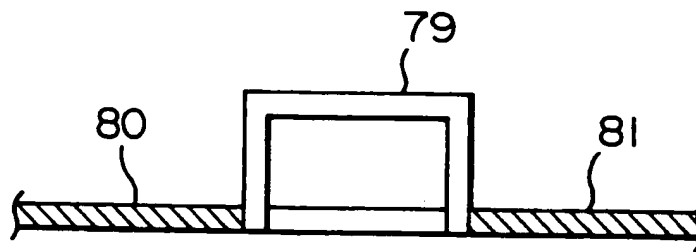


FIG. 5C

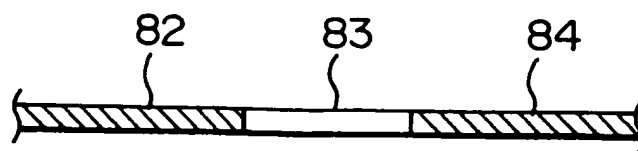


FIG. 6A

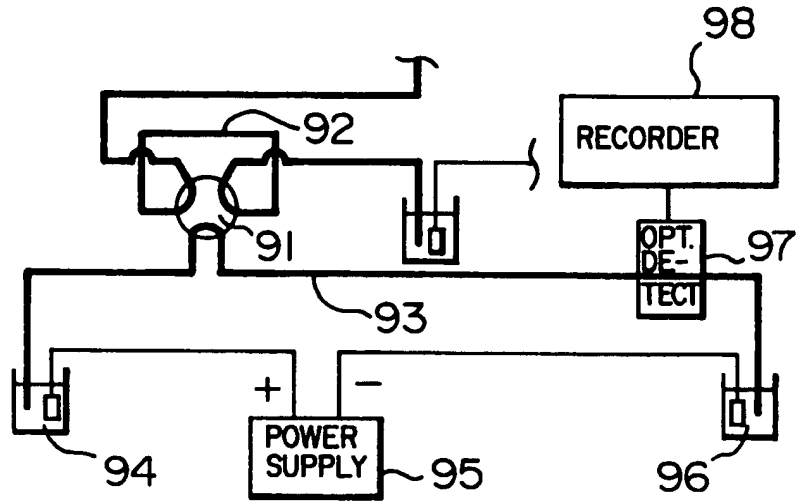


FIG. 6B

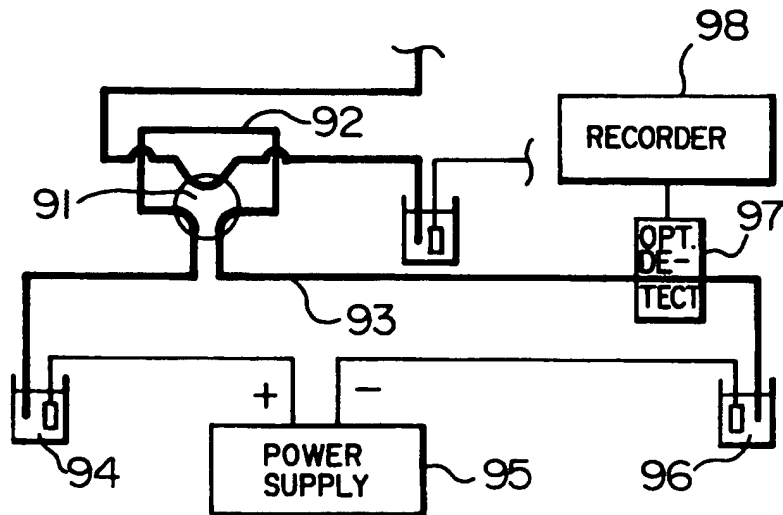


FIG. 8A

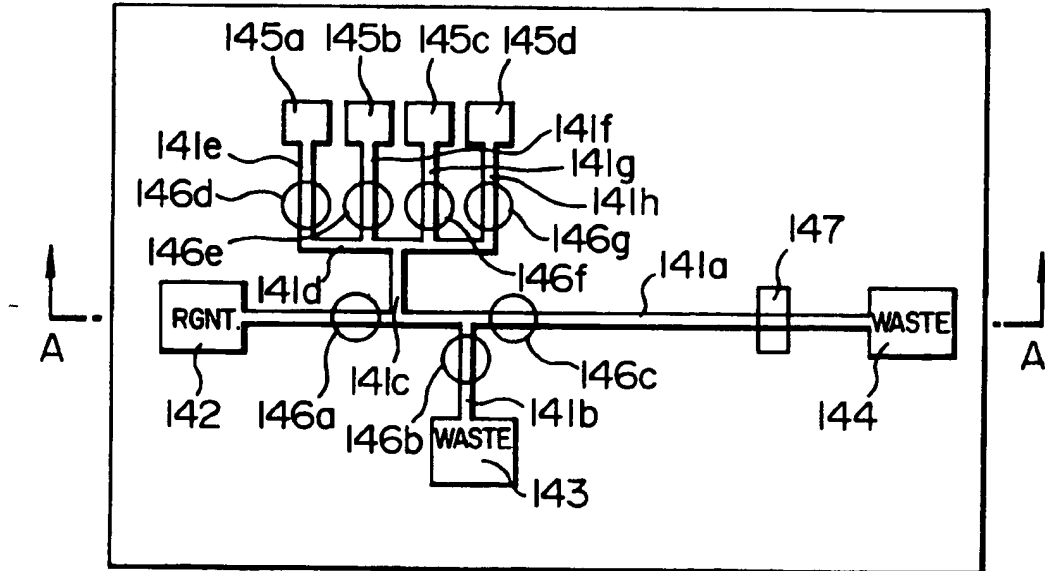


FIG. 8B

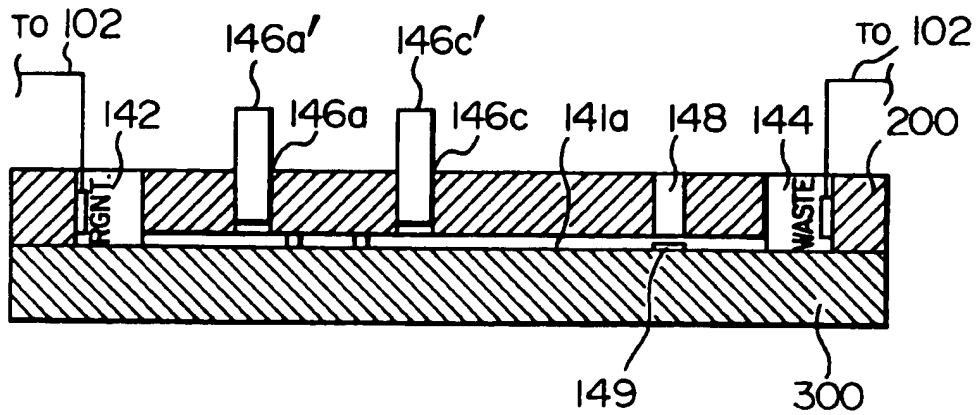


FIG. 9A

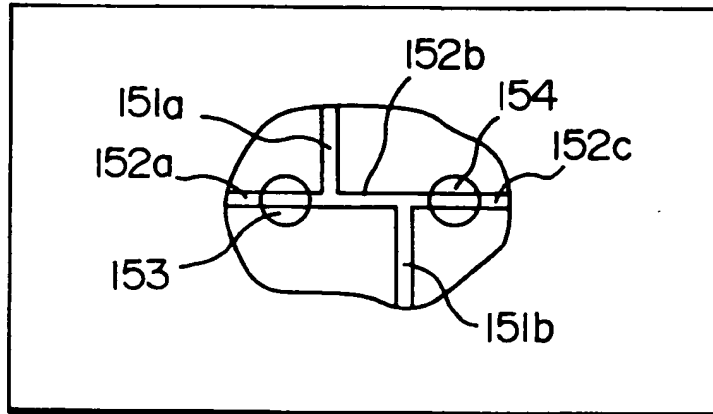
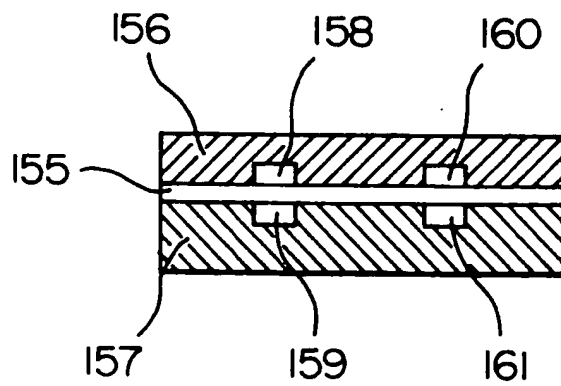


FIG. 9B





European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 30 1820

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
X	DE-A-41 05 107 (CIBA-GEIGY AG)	1,2,4-7, 9,10	G01N35/08 G01N1/00 B01L3/00
Y	* column 5, line 28 - column 8, line 28 * ---	3,8	
X	US-A-4 908 112 (PACE)	1,2,4,7, 9,10	
Y	* column 5, line 35 - column 10, line 55 * ---	11,12	
Y	EP-A-0 081 116 (HITACHI LTD) * figures 1,2 *	3,8	
Y	WO-A-91 15750 (CARRIMED LTD) * page 2, line 18 - line 27 * ---	11,12	
A	DE-A-35 23 558 (ERNO RAUMFAHRTECH GMBH) * abstract *	13,14	
A	IEEE TRANSACTIONS ON ELECTRON DEVICES, vol.ED-26, no.12, December 1979, NEW YORK US pages 1880 - 1886 TERRY ET AL. 'A gas chromatographic air analyzer fabricated on a silicon wafer' * figures 1,4 *	3,8,11	
A	US-A-5 100 627 (BUICAN ET AL.) * column 4, line 9 - line 23 * -----	3,8,11	G01N B01L
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 13 June 1994	Examiner Bindon, C
CATEGORY OF CITED DOCUMENTS		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application I : document cited for other reasons & : member of the same patent family, corresponding document	
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document			

EPO FORM LSO 01.82 (P0401)

THIS PAGE BLANK (USPTO)